

Skin prick test and serological analysis with recombinant group 2 allergens of the dust mites *L. destructor* and *T. putrescentiae*

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Summary

Background The dust mites *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* are important sources of allergen in farming environments. The major allergens of the dust mites *L. destructor* and *T. putrescentiae* have been cloned and expressed as recombinant proteins.

Objective To evaluate the use of recombinant group 2 allergens of *L. destructor* (rLep d 2) and *T. putrescentiae* (rTyr p 2) in skin prick test (SPT), and serological analysis in sensitized and non-sensitized farmers chronically exposed to dust mites.

Methods Skin prick test with rLep d 2, rTyr p 2 and the corresponding commercial extracts was performed in 44 farmers sensitized to *L. destructor* and/or *T. putrescentiae*, and 38 control farmers. IgE and IgG subclass antibodies to the recombinant allergens were analysed by RAST and ELISA, respectively.

Results Out of the 44 subjects positive in SPT to *L. destructor* and/or *T. putrescentiae* extract, 26 (59%) displayed a positive SPT to one or the other of the recombinant allergens, whereas 21 (48%) were positive to both. Significant correlations were registered between the sizes of the weals induced by rLep d 2 and rTyr p 2 and the corresponding RAST values ($P < 0.001$). A majority of subjects positive in SPT to the recombinant allergens had detectable IgG4 antibodies, and the levels were significantly higher in the dust mite sensitized group than in the controls ($P < 0.05$). No such differences were found in the IgG1 values ($P > 0.05$). The results obtained with rLep d 2 and rTyr p 2 correlated relatively well with each other with respect to SPT, RAST and IgG4, suggesting that the allergens have similar or shared IgE epitopes. All the control subjects had a negative SPT and RAST to rLep d 2 and rTyr p 2.

Conclusion Recombinant group 2 allergens from the dust mite *L. destructor* and *T. putrescentiae* represent useful tools for diagnosis of dust mite allergy.

Keywords: *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, recombinant allergens, rLep d 2, rTyr p 2, SPT, RAST, IgG

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Introduction

Clinical history, skin tests (skin prick test (SPT) or intradermal skin test) and different *in vitro* methods are the tools

for diagnosis of allergic diseases. Until recently, the only allergenic substances available for skin tests, serological assays and immunotherapy have been crude allergen extracts that contain many different components. These extracts are difficult to standardize and the batch-to-batch variation may be considerable. Furthermore, the use of crude allergen preparations may provoke the development

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of sensitization against additional allergenic proteins in the extract [1].

During the last decade, molecular biology techniques have been used for the production of pure recombinant allergens. A number of recombinant allergens from pollens, moulds, animal danders and mites have been produced [2] and several of them demonstrate IgE reactivity, both *in vivo* and *in vitro*. Recombinant allergens are easy to standardize and can therefore improve the diagnosis of allergic diseases.

The dust mites *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* are a major cause of allergic disease among farmers, and are even more important than allergens from animal danders and pollens [3,4]. In addition, these mites have also been found to cause sensitization in urban environments [5,6]. Immunological characterization of *L. destructor* and *T. putrescentiae* extracts has demonstrated several proteins that can give rise to an IgE response [7–9]. The group 2 allergens, Lep d 2 and Tyr p 2, with a molecular mass of approximately 15–16 kDa, are major allergens in these species [7,8]. These allergens have been cloned, expressed as recombinant proteins (rLep d 2 and rTyr p 2) and demonstrated to possess IgE reactivity *in vitro* [10–13]. They show around 40% identity with group 2 allergens from house dust mites [12,14].

The aim of this study was to investigate the clinical and serological relevance of rLep d 2 and rTyr p 2 in a group of sensitized and non-sensitized farmers chronically exposed to dust mites.

Materials and methods

Study population

All individuals in this study participated in an investigation which included SPT, a medical interview and venous blood sampling for serological analysis. The study comprised 44 farmers (mean age 44 years, range 31–66) who were positive in SPT to commercial extracts of the dust mites *L. destructor* ($n=41$) and/or *T. putrescentiae* ($n=25$). Forty-one subjects reported respiratory symptoms and three were asymptomatic. The control groups were composed of 19 farmers sensitized to common aeroallergens others than dust mites (mean age 43 years, 33–64) and 19 non-atopic farmers (mean age 49 years, 36–66). In the two control groups, 14 reported airway symptoms and 24 were asymptomatic. None of the farmers had previously received immunotherapy, and treatment with antihistamine was withdrawn at least 5 days prior to the investigation. None of the subjects suffered from clinically active atopic dermatitis. The examination was performed on Gotland, an island in the Baltic Sea, in April 1998.

Recombinant allergen Lep d 2 was produced both in a prokaryotic (rLep d 2 [*E. coli*]) and a eukaryotic (rLep d 2

[baculovirus]) expression system. The Lep d 2.01 isoform (Lep d 1.0101 in [10]) was expressed in *E. coli* with a C-terminal His₆ tag (six histidine) and purified using His-tag affinity chromatography [11]. The Lep d 2.02 isoform (Lep d 1.02 in [10]) was expressed in insect cells using a baculovirus expression system (Invitrogen, Leek, The Netherlands) and purified by gel filtration and reverse phase HPLC as described [11]. rTyr p 2 was expressed in *E. coli* with a C-terminal His₆ tag and recovered by affinity purification [12]. The purity of the recombinant proteins was verified by SDS-PAGE under reducing conditions, giving single protein bands. Allergen concentrations were determined as described elsewhere [11] and the recombinant allergens were diluted in 0.9% NaCl to 100, 10 and 1 µg/mL, aliquoted, and stored at –20 °C until use.

Commercial extracts of *L. destructor* and *T. putrescentiae* (10 000 BU/mL) were obtained from ALK, Hørsholm, Denmark.

Skin testing

The SPT was performed in duplicate on the volar aspect of the forearms by a trained nurse. Each drop consisted of approximately 15 µL of the solution and was pricked with a sterile lancet (ALK). Histamine dihydrochloride 10 mg/mL (ALK) and 0.9% NaCl were used as positive and negative controls, respectively. SPT results were recorded after 15 min, and the weals were outlined with a fine-tipped pen and transferred to paper with transparent tape. The weal diameter was calculated according to the formula $(D1 + D2)/2$, where D1 represents the largest longitudinal diameter and D2 the maximal transverse diameter. The mean value of the duplicate weals was calculated and considered positive if at least 2+ and 3 mm in diameter [15].

Determination of allergen specific antibodies

IgE antibodies to rLep d 2 and rTyr p 2 IgE antibodies to rLep d 2 (*E. coli*) and rTyr p 2 were analysed by Pharmacia CAP system, RAST® FEIA (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). ImmunoCAPs with rLep d 2 and rTyr p 2 were prepared as described elsewhere [13]. A positive RAST was defined as ≥ 0.35 kU/L.

IgG antibodies to rLep d 2 and rTyr p 2 Allergen-specific IgG1 and IgG4 subclass antibodies were measured by ELISA as previously described [16,17]. Briefly, microtitre plates (Maxisorp, Nunc, Denmark) were coated with purified rLep d 2 (*E. coli*) and rTyr p 2 allergen (5 µg/mL). All serum samples were assayed at a final 10-fold (IgG4) or 50-fold (IgG1) dilution following pre-incubation with the corresponding allergen (5 µg/mL) or diluent alone. The net results in optical density at 405 nm were used to

interpolate the concentration of IgG1 and IgG4 antibodies from standard curves constructed by threefold dilutions of calibrated concentrations of chimeric IgG1 and IgG4 anti-NIP hapten antibodies [17] run in parallel using BSA-NIP conjugate (10 µg/mL) as coating antigen. Taking the serum dilution into account, and considering a net optical density result of 0.020 as cut-off, the sensitivity in serum was 4.0 AU (arbitrary units)/mL and 0.30 AU/mL for IgG1 and IgG4 antibodies, respectively. Serum samples from all 44 SPT-positive farmers, eight atopic controls and eight non-atopic controls were analysed.

SDS-PAGE and immunoblotting

SDS-PAGE was performed using the Mini-Protean® equipment (Bio-Rad, Richmond, CA, USA). Electrophoresis to PVDF membrane (Immobilon™, Millipore, Bedford, MA, USA) was performed in the Hoefer® Mighty Small™ Unit (Amersham Pharmacia Biotech, Uppsala, Sweden) according to the operating instructions. Extracts of *L. destructor* and *T. putrescentiae* (15 µg/mm gel) were separated under non-reducing conditions in homogenous 15% slab gels. Immunodetection with human sera was performed as described earlier [8].

Statistical analysis

Ages are given as mean values with range and serological results, as medians with range. Correlation coefficients were determined using Spearman rank correlation analysis. Differences in serological results between groups were calculated with the Mann–Whitney *U*-test. *P*-values <0.05 were considered significant.

Ethical considerations

The study was approved by the Ethics Committee of the Karolinska Institute, Stockholm.

Results

Skin prick test reactivity

A dose-dependent relationship between the allergen concentration and the proportion of positive tests was noted for rLep d 2 (*E. coli*) (none reacted at 1 µg/mL, 16 at 10 µg/mL and 24 at 100 µg/mL) as well as for rLep d 2 (baculovirus) (two at 1 µg/mL, 21 at 10 µg/mL and 23 at 100 µg/mL), but not for rTyr p 2 (one at 1 µg/mL and 10 µg/mL, and 23 at 100 µg/mL). Since the results obtained at 100 µg/mL for rLep d 2 (*E. coli*) were similar to those obtained using rLep d 2 (baculovirus), only the results with rLep d 2 (*E. coli*) are presented for simplicity. In the following, all SPT results

refer to data obtained with the highest concentration (100 µg/mL) of the recombinant allergens.

rLep d 2

Among the 41 subjects positive in SPT to *L. destructor* extract, 24 (59%) were SPT positive to rLep d 2 (Table 1 and Fig. 1). There was no significant correlation between the weal size for rLep d 2 and those produced by the *L. destructor* extract ($n = 44$, $r_s = 0.31$). All of the 24 subjects with positive SPT against rLep d 2 reported respiratory symptoms, compared with 38 out of the 41 subjects positive to *L. destructor* extract. None of the 38 subjects in the two control groups had a positive SPT to rLep d 2.

rTyr p 2

Of the 25 subjects positive to extract of *T. putrescentiae*, 14 (56%) displayed a positive SPT to rTyr p 2 (Table 1 and Fig. 1). Interestingly, there were nine subjects who had positive SPT to rTyr p 2 but not to *T. putrescentiae* extract. The weal sizes for rTyr p 2 and *T. putrescentiae* extract were not significantly correlated ($n = 44$, $r_s = 0.35$). A history of respiratory symptoms was reported by all subjects positive to rTyr p 2 and by 23 out of 25 subjects positive to *T. putrescentiae*. No positive SPT to rTyr p 2 was found in the two control groups.

Of the 44 subjects positive in SPT to *L. destructor* and/or *T. putrescentiae* extract, 26 (59%) displayed a positive SPT to one or the other of the recombinant allergens, whereas 21 (48%) were positive to both. A significant correlation was registered between the sizes of the weals induced by rLep d 2 and rTyr p 2 ($n = 44$, $r_s = 0.76$, $P < 0.001$).

Immunoglobulin reactivity to rLep d 2 and rTyr p 2

rLep d 2 All but one of the 24 subjects that were positive in SPT to rLep d 2 were also positive in RAST to rLep d 2 (3.5 kU/L, 0.52–46) (Table 1). There was a significant correlation between the weal sizes and the corresponding RAST values ($n = 44$, $r_s = 0.92$, $P < 0.001$). Of the 17 subjects having a negative SPT to the recombinant allergen and a positive SPT to *L. destructor* extract, none had a positive RAST to rLep d 2. However, in immunoblotting, two of these recognized a 15 kDa band in *L. destructor* extract, probably corresponding to natural Lep d 2. No positive RAST were found in the two control groups.

Detectable levels of IgG1 antibodies to rLep d 2 were found among all 24 subjects SPT positive to rLep d 2 (17 AU/mL, 5.1–80) (Table 1). The corresponding figure for IgG4 was 21 (4.7 AU/mL, 0.37–54). In the 16 controls analysed, detectable IgG1 was found among 12 (13 AU/mL, 4.3–39) and low levels of IgG4 among three subjects (0.60, 0.90 and 1.1 AU/mL). Levels of IgG4 antibodies to rLep d 2 were significantly higher among the dust mite sensitized

Table 1. SPT and serological results for 44 farmers SPT-positive to *L. destructor* and/or *T. putrescentiae*

Subj. no.	SPT				RAST		ELISA				Immunoblotting	
	L. d	T. p	rLep d 2	rTyr p 2	IgE rLep d 2	IgE Tyr p 2	IgG1 rLep d 2	IgG1 rTyr p 2	IgG4 rLep d 2	IgG4 rTyr p 2	Native rLep d 2	Native rTyr p 2
1	+	+	+	+	46	4.1	16	29	54	0.54	+	+
2	+	+	+	+	15	7.2	23	0	3.8	0	+	+
3	+	+	+	+	8.5	5.3	65	54	11	1.7	+	+
4	+	+	+	+	7.2	6.8	8.0	6.9	9.8	6.3	+	+
5	+	+	+	+	6.6	8.0	80	0	18	2.9	+	+
6	+	+	+	+	5.8	3.6	7	0	1.8	2.2	+	+
7	+	+	+	+	4.7	3.4	10.2	26	16	8.7	+	+
8	+	+	+	+	3.9	1.4	9.1	17	23	7.5	+	+
9	+	+	+	+	3.8	2.5	21	78	4.7	2.8	+	+
10	+	+	+	+	3.5	2.0	22	51	3.0	2.5	+	+
11	+	+	+	+	3.0	2.0	6.5	18	5.5	11	+	+
12	+	+	+	+	2.8	1.1	19	0	3.3	0.85	+	+
13	+	+	+	+	1.1	0.92	6.3	0	0	0	+	+
14	+	-	+	+	23	15	23	18	38	13	+	+
15	+	-	+	+	3.6	0	18	11	0.64	0.32	+	-
16	+	-	+	+	2.6	1.8	19	52	0.64	0.36	+	+
17	+	-	+	+	1.8	0.93	31	69	7.6	2.8	+	+
18	+	-	+	+	1.8	2.2	5.1	0	6.9	11	+	+
19	+	-	+	+	1.4	0	26	18	0.81	0.58	+	+
20	+	-	+	+	1.0	0.70	6.1	33	0.37	0.43	+	+
21	+	-	+	+	0.67	0.79	21	60	0	0.34	+	+
22	+	-	+	-	0.84	0	12	31	1.4	0	+	-
23	+	-	+	-	0.52	0	10	19	3.8	0	-	-
24	+	-	+	-	0	0	7.8	37	0	0	-	-
25	+	+	-	+	0	0	70	0	0	0.59	+	-
26	+	-	-	+	0	2.1	19	18	2.6	0.98	+	+
27	+	+	-	-	0	0	0	0	0.49	5.3	-	-
28	+	+	-	-	0	0	25	8	0	7.6	-	-
29	+	+	-	-	0	0	66	51	0	3.9	-	-
30	+	+	-	-	0	0	4.5	16	0	0.43	-	-
31	+	+	-	-	0	0	26	47	0	0	-	-
32	+	+	-	-	0	0	0	7.6	0	0	-	-
33	+	+	-	-	0	0	0	47	0	0	-	-
34	+	+	-	-	0	0	0	0	0	0	-	-
35	+	-	-	-	0	0	7.7	25	0.51	0	-	-
36	+	-	-	-	0	0	5.4	35	0.50	0	-	-
37	+	-	-	-	0	0	22	29	0.31	0	-	-
38	+	-	-	-	0	0	21	30	0	0	-	-
39	+	-	-	-	0	0	0	54	0	0	-	-
40	+	-	-	-	0	0	0	0	0	0	-	-
41	+	-	-	-	0	0	0	0	0	0	-	-
42	-	+	-	-	0	0	4.7	8.5	0	0.66	-	-
43	-	+	-	-	0	0	5.1	64	0	0	-	-
44	-	+	-	-	0	0	64	34	0	0	-	-

L. d = *L. destructor* extract, T. p = *T. putrescentiae* extract, rLep d 2 = recombinant Lep d 2, rTyr p 2 = recombinant Tyr p 2
 (+) = positive SPT/immunoblotting and (-) = negative SPT/immunoblotting. Serological values below cut-off (IgE <0.35 kU/L, IgG1 <4 AU/mL and IgG4 <0.30 AU/mL) are assigned 0.

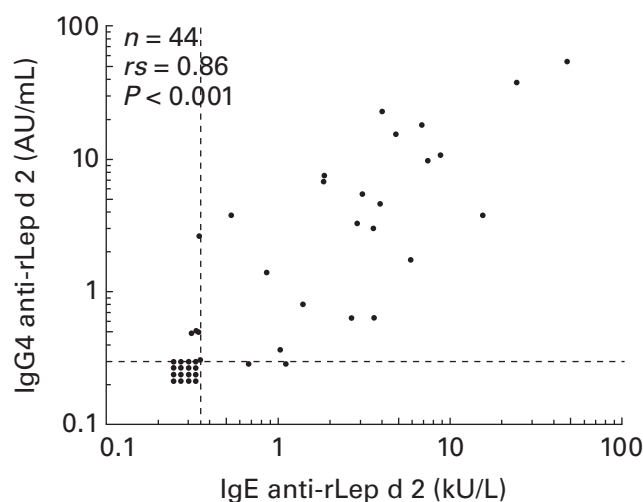


Fig. 1. The correlation between IgE and IgG4 antibodies to rLep d 2. The correlation coefficient $r_s = 0.86$ was calculated on mite-sensitized farmers ($n = 44$). The dotted lines represent the detection limits of the assays. $P < 0.001$.

subjects compared with the controls ($P < 0.01$). No such difference was observed between the IgG1 values ($P > 0.05$). Moreover, the concentration of IgE antibodies to rLep d 2 correlated significantly with the corresponding IgG4 values ($n = 44$, $r_s = 0.86$, $P < 0.001$) in the dust mite sensitized farmers (Fig. 1). The correlation with the IgG1 values was $r_s = 0.33$ ($n = 44$, $P < 0.05$).

rTyr p 2 Twenty out of 23 subjects with a positive SPT to rTyr p 2 were also positive in RAST to rTyr p 2 (2.1 kU/L, 0.70–15) (Table 1). The size of the SPT weals induced by rTyr p 2 correlated with the corresponding RAST values ($n = 44$, $r_s = 0.91$, $P < 0.001$). Of the nine subjects positive in SPT to rTyr p 2 but not to *T. putrescentiae* extract, seven had a positive RAST to rTyr p 2 and in immunoblotting, eight recognized a 16 kDa band in *T. putrescentiae* extract, probably corresponding to natural Tyr p 2. Among the subjects positive in SPT only to *T. putrescentiae* extract, none had a positive RAST to rTyr p 2 or recognized natural Tyr p 2 in immunoblotting. No positive RAST were found among the controls.

Among the 23 subjects positive in SPT to rTyr p 2, IgG1 and IgG4 antibodies to rTyr p 2 were detectable in 16 (27 AU/mL, 6.9–78) and 21 (2.2 AU/mL, 0.32–13) subjects (Table 1). Eleven subjects out of 16 in the control groups had detectable IgG1 antibodies to rTyr p 2 (22 AU/mL, 6.9–64) and three, to IgG4 (0.35, 1.0 and 16 AU/mL). The control having the highest IgG4 level (16 AU/mL) of all subjects had no IgE antibodies to natural Tyr p 2 in *T. putrescentiae* extract as determined by immunoblotting (data not shown). The IgG4 values among the sensitized farmers were

significantly higher than in the control subjects ($P < 0.05$), while no difference was found in the IgG1 values ($P > 0.05$). There was a significant correlation between IgE and IgG4 to rTyr p 2 ($n = 44$, $r_s = 0.60$, $P < 0.001$) but not between IgE and IgG1 ($n = 44$, $r_s = -0.07$), nor between the two IgG subclasses ($n = 44$, $r_s = -0.13$).

The concentrations of IgE antibodies to rLep d 2 and rTyr p 2 correlated significantly ($n = 44$, $r_s = 0.86$, $P < 0.001$), as did the concentrations of IgG4 ($n = 44$, $r_s = 0.60$, $P < 0.001$). The correlation between the IgG1 values to the two recombinant allergens was not significant ($n = 44$, $r_s = 0.24$).

Discussion

In this study, the recombinant dust mite allergens, rLep d 2 and rTyr p 2, were evaluated both *in vivo* and *in vitro* among farmers with positive SPT to the corresponding commercial extracts, as well as among non-mite-sensitized farmers. Only a minor difference in skin test reactivity was obtained between rLep d 2 produced in a prokaryotic (*E. coli*) and in a eukaryotic (baculovirus) expression system. It has been demonstrated previously that rLep d 2 produced in these two expression systems shows similar IgE binding properties *in vitro* [11]. The present investigation confirms, in a large group of subjects, that the *in vivo* reactivity of rLep d 2 (*E. coli*) and rLep d 2 (baculovirus) are also very similar as measured by SPT. The minor differences observed between the rLep d 2 allergens may be due to folding properties in the two expression systems, or to the molecular form (His-tag and isoforms) of the two proteins. Therefore, no further distinction will be made between rLep d 2 (*E. coli*) and rLep d 2 (baculovirus) and both will be referred to as rLep d 2.

Of the mite-sensitized farmers identified with *L. destructor* and *T. putrescentiae* extracts, 59% and 56% were identified, respectively, with rLep d 2 and rTyr p 2. A combination of rLep d 2 and rTyr p 2 only detected two more subjects. It should be noted that SPT with rTyr p 2 gave a positive test in nine farmers who were negative in SPT to *T. putrescentiae* extract; eight were also SPT positive to rLep d 2. Many farmers have IgE antibodies to several dust mite species [4] as a possible consequence of exposure to different mite species or cross-reactivity. Both *L. destructor* and *T. putrescentiae* are part of the mite fauna on Gotlandic farms, but *L. destructor* is the predominant species [18]. The fairly high correlation coefficients obtained between rLep d 2 and rTyr p 2 with respect to SPT, RAST and IgG4 values probably reflect the fact that group 2 allergens from different mite species share, or have similar IgE epitopes [8]. However, the existence of subjects having IgE [13] or IgG4 antibodies exclusively to rLep d 2 or rTyr p 2 supports the presence of unique epitopes in both mite species [8]. The finding that eight farmers were negative in SPT to *T. putrescentiae* extract but recognized

natural Tyr p 2 in immunoblotting suggests either that there were insufficient amounts of Tyr p 2 in the commercial *T. putrescentiae* extract, or that there was a high degree of cross-reactivity with rLep d 2. Seven of these farmers also had, in addition to positive SPT and RAST to rTyr p 2, higher IgE levels to rLep d 2 than to rTyr p 2.

In accordance with previous reports on immunoglobulin reactivity to aeroallergens, we did not observe any differences in the IgG1 levels between mite-sensitized and non-mite-sensitized farmers [19,20]. Detectable levels of allergen specific IgE and IgG4 among individuals chronically exposed to allergens, e.g. farmers sensitized to *L. destructor*, as well as among children sensitized to *Dermatophagoides pteronyssinus*, have been demonstrated [19,21]. The present study confirms a co-existence of IgE and IgG4 to Lep d 2 as well as to Tyr p 2 among subjects positive in SPT to the recombinant allergens. However, in contrast to Tame *et al.* [19], we found significant correlations between IgE and IgG4 values, to rLep d 2 and rTyr p 2, as was also recently observed for the recombinant birch pollen allergen rBet v 1 [22].

One of the non-atopic asymptomatic controls had, in spite of negative SPT, RAST, immunoblotting and IgG1, a markedly elevated IgG4 to rTyr p 2 (16 AU/mL). Similar findings have earlier been reported where, despite negative IgE, some subjects exhibited positive levels of house dust mite specific IgG4 [23,24]. In addition, there were also three farmers positive in SPT to rTyr p 2, and five negative, that displayed IgG4 antibodies but were negative in RAST. These findings might be related to a chronic allergen exposure [25].

Not surprisingly, the number of subjects with positive SPT to the recombinant preparations was lower than that obtained with the whole mite extracts containing several allergens. The same observation was reported earlier both in studies with recombinant house dust mites allergens (rDer p 2, rDer p 5 and rDer p 7) and recombinant allergen of *A. fumigatus* (rAsp/Ia) [26,27]. These findings illustrate the need to identify additional allergens to optimize the diagnosis further. However, in some cases, sensitization against one major allergen occurs, as for birch pollen, where more than 90% of birch pollen-sensitized subjects can be identified by IgE against the recombinant major allergen, rBet v 1 [22,28,29].

In summary, this study shows the ability of recombinant Lep d 2 and Tyr p 2 to induce positive SPT in subjects sensitized to dust mite species and that the results correlate with the serological data. However, the allergen panorama of *L. destructor* and *T. putrescentiae* needs to be investigated further and supplementary recombinant allergens produced. Probably, by adding a few more crucial allergens, a higher diagnostic accuracy can be achieved. It will be possible to determine each patient's unique allergen profile

and create individually tailored allergen vaccines for immunotherapy.

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References

- 1 van Ree R, van Leeuwen WA, Dieges PH *et al.* Measurement of IgE antibodies against purified grass pollen allergens (Lol, p 1, 2, 3 and 5) during immunotherapy. *Clin Exp Allergy* 1997; 27:68–74.
- 2 Scheiner O, Kraft D. Basic and practical aspects of recombinant allergens. *Allergy* 1995; 50:384–91.
- 3 van Hage-Hamsten M, Johansson SGO, Zetterström O. Predominance of mite allergy over allergy to pollens and animal danders in a farming population. *Clin Allergy* 1987; 17:417–23.
- 4 Kronqvist M, Johansson E, Pershagen G, Johansson SGO, van Hage-Hamsten M. Increasing prevalence of asthma over 12 years among dairy farmers on Gotland, Sweden: storage mites remain dominant allergens. *Clin Exp Allergy* 1999; 29:35–41.
- 5 Ebner C, Feldner H, Ebner H, Kraft D. Sensitization to storage mites in house dust mite (*Dermatophagoides pteronyssinus*) allergic patients. Comparison of a rural and an urban population. *Clin Exp Allergy* 1994; 24:347–52.
- 6 van der Heide S, Niemeijer NR, Hovenga H, de Monchy JGR, Dubois AEJ, Kauffman HF. Prevalence of sensitization to the storage mites *Acarus siro*, *Tyrophagus putrescentiae*, and *Lepidoglyphus destructor* in allergic patients with different degrees of sensitization to the house-dust mite *Dermatophagoides pteronyssinus*. *Allergy* 1998; 53:426–30.
- 7 Johansson E, van Hage-Hamsten M, Johansson SGO. Demonstration of allergen components in the storage mite *Lepidoglyphus destructor* by an immunoblotting technique. *Int Arch Allergy Appl Immunol* 1988; 85:8–13.
- 8 Johansson E, Johansson SGO, van Hage-Hamsten M. Allergenic characterization of *Acarus siro* and *Tyrophagus putrescentiae* and their crossreactivity with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1994; 24:743–51.
- 9 Arlian LG, Vyszynski-Moher DL, Johansson SGO, van Hage-Hamsten M. Allergenic characterization of *Tyrophagus putrescentiae* using sera from occupationally exposed farmers. *Ann Allergy Asthma Immunol* 1997; 79:525–9.

- 10 Schmidt M, Olsson S, van der Ploeg I, van Hage-Hamsten M. cDNA analysis of the mite allergen Lep d 1 identifies two different isoallergens and variants. *FEBS Lett* 1995; 370:11–4.
- 11 Olsson S, van Hage-Hamsten M, Whitley P et al. Expression of two isoforms of Lep d 2, the major allergen of *Lepidoglyphus destructor*, in both prokaryotic and eukaryotic systems. *Clin Exp Allergy* 1998; 28:984–91.
- 12 Eriksson TLJ, Johansson E, Whitley P, Schmidt M, Elsayed S, van Hage-Hamsten M. Cloning and characterisation of a group II allergen from the dust mite *Tyrophagus putrescentiae*. *Eur J Biochem* 1998; 251:443–7.
- 13 Johansson E, Eriksson TLJ, Olsson S et al. Evaluation of specific IgE to the recombinant group 2 mite allergens Lep d 2 and Tyr p 2 in the Pharmacia CAP System. *Int Arch Allergy Immunol*, 1999; in press.
- 14 Smith W, Mills KL, Hazell LA, Hart BJ, Thomas WR. Molecular analysis of the group 1 and 2 allergens from the house dust mite, *Euroglyphus maynei*. *Int Arch Allergy Immunol* 1999; 118:15–22.
- 15 Aas K, Belin L. Standardization of diagnostic work in allergy. *Int Arch Allergy* 1973; 45:57–60.
- 16 Magnusson CGM. Major differences in specificity among naturally occurring human IgG-subclass anti-IgE autoantibodies. *J Allergy Clin Immunol* 1995; 95:1011–9.
- 17 Magnusson CGM, Nelson DF, Magnusson MAJ. Disproportional distribution of isotype and non-isotype-specific IgG subclass anti-IgE autoantibodies in human cord serum. *Int Arch Allergy Immunol* 1996; 110:31–40.
- 18 Boström S, Johansson E, Härfast B et al. Characterization of the mite fauna (Acari) in Swedish barn dust. *Internat J Acarol* 1997; 23:127–32.
- 19 Tame A, Sakiyama Y, Kobayashi I, Terai I, Kobayashi K. Differences in titres of IgE, IgG4 and other IgG subclass anti-Der p 2 antibodies in allergic and non-allergic patients measured with recombinant allergen. *Clin Exp Allergy* 1996; 26:43–9.
- 20 Kemeny M, Urbanek R, Ewan P et al. The subclass of IgG antibody in allergic disease: II. The IgG subclass of antibodies produced following natural exposure to dust mite and grass pollen in atopic and non-atopic individuals. *Clin Exp Allergy* 1989; 19:545–9.
- 21 Johansson E, Härfast B, Johansson SGO, van Hage-Hamsten M. IgG1 and IgG4 antibody responses to the dust mite *Lepidoglyphus destructor* in a naturally exposed farming population. *Allergy* 1995; 50:473–7.
- 22 van Hage-Hamsten M, Kronqvist M, Zetterström O et al. Skin test evaluation of genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1: Results obtained with a mix of two recombinant Bet v 1 fragments and rBet v 1 trimer in a Swedish population before the birch pollen season. *J Allergy Clin Immunol*, 1999; 104:969–77.
- 23 Lin KL, Wang SY, Hsieh KH. Analysis of house dust mite-specific IgE, IgG4, and IgG antibodies during immunotherapy in asthmatic children. *Ann Allergy* 1991; 67:63–9.
- 24 Kobayashi I, Sakiyama Y, Tame A, Kobayashi K, Matsumoto S. IgE and IgG4 antibodies from patients with mite allergy recognize different epitopes of *Dermatophagoides pteronyssinus* group II antigen (Der p 2). *J Allergy Clin Immunol* 1996; 97:638–45.
- 25 Aalberse RC, van der Gaag R, van Leeuwen J. Serological aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* 1983; 130:722–6.
- 26 Lynch NR, Thomas WR, Garcia NM et al. Biological activity of recombinant Der p 2, Der p 5 and Der p 7 allergens of the house-dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Immunol* 1997; 114:59–67.
- 27 Moser M, Cramer R, Brust E, Suter M, Menz G. Diagnostic value of recombinant *Aspergillus fumigatus* allergen I/a for skin testing and serology. *J Allergy Clin Immunol* 1994; 93:1–11.
- 28 Menz G, Dolecek C, Schönheit-Kenn U et al. Serological and skin-test diagnosis of birch pollen allergy with recombinant Bet v I, the major birch pollen allergen. *Clin Exp Allergy* 1996; 26:50–60.
- 29 Pauli G, Oster JP, Deviller P et al. Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: Diagnostic value for birch pollen and associated allergies. *J Allergy Clin Immunol* 1996; 97:1100–9.